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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
BLAND, LAYLA D	

ART UNIT	PAPER NUMBER
1623	

MAIL DATE	DELIVERY MODE
04/10/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/587,878	RAAIJMAKERS ET AL.	
	Examiner	Art Unit	
	LAYLA BLAND	1623	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 July 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-11* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-11* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☒ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

This application is a national stage entry of International Application No. PCT/BE05/00011, filed January 28, 2005, which claims priority to European Application No. 04075280.0 filed on January 30, 2004. The certified copy of the priority has not been filed with the instant Application.

Claims 1-11 are pending and are examined on the merits herein.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3, 5, 10, and 11 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 3 depends from claim 1 and recites the limitation "wherein the molar ratio of halogenoalkylcarboxylate : inulin is in the range of from 1.5 to 4.5." In the method of claim 1, halogenoalkylcarboxylate is added in step (a) and again in step (c). It is unclear whether the molar ratio in claim 3 refers to the initial reaction or if the molar ratio of claim 3 is intended to be the final ratio.

Claim 5 ultimately depends from claim 1 and recites the limitation "wherein the pH of the reaction mixture is in the range of from 9.5 to 11.5." Claim 1 recites two different pH limitations at different steps in the method. It is unclear at which point in the method of claim 1 the pH should be 9.5 to 11.5.

Claims 10 and 11 depend from claim 1 and recite limitations wherein the aqueous medium in step (a) optionally contains inulin at specific weight percentages. In claim 1, inulin is added to the mixture in step (b), not in step (a). It is unclear whether claims 9 and 10 intend for inulin to be present in step (a) in the recited amounts, and then additional inulin is added in step (b), or if claims 9 and 10 are intended only to limit the amount of inulin which is added in step (b).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Verraest et al. (Carbohydrate Research 271 (1995) 101-112) in view of Raehse et al. (US 4,507,474, March 26, 1985).

Verraest et al. teach a process for preparing carboxymethyl inulin. Inulin is dissolved in water and NaOH and monochloroacetic acid or its sodium salt are added, followed by heating [pages 102 and 103, Methods (a) and (b)]. Reactions were carried out for 60-240 minutes at 55°C, 75°C, and 95°C with a molar ratio of inulin: MCA: NaOH of 1:2:4.2 [page 108, Figure 4 and Table 3]. When 20 (3.4 g) mmol of inulin was used, water content of the reaction was 5-25 mL (about 14-68% inulin by weight) [page 109, Table 4].

Verraest et al. do not teach a process wherein additional halogenoalkylcarboxylate and alkali are added during the heating step.

Raehse et al. teach a process for preparing highly substituted carboxyalkyl celluloses comprising up to 3 stages [see abstract]. During each reaction stage, the carboxyalkylating agent is used in a quantity of no more than 2.5 moles per mole of anhydroglucose unit and the alkalizing agent is used in a quantity of no more than 5 moles per mole of anhydroglucose unit [column 2, lines 14-19]. Satisfactory results are usually obtainable with only 2 reaction stages [column 2, lines 36-40]. Etherification is normally carried out at temperatures in the range of about 30-85°C, with the exception of the first stage which is carried out at temperatures in the range of about 10-30°C [column 3, lines 8-14]. Preferred carboxylating agents include the sodium salt of monochloroacetic acid [column 4, lines 61-64]. The reaction medium is aqueous [column 8, Examples]. The quantity of carboxyalkylating agent used in each step can be 1.0-2.2 moles and the quantity of alkalizing agent used in each step can be about 2-4 moles [claims 3-6].

It would have been obvious to one of ordinary skill in the art at the time the invention was made to carry out the method of Varraest, and to include a second reaction stage wherein more MCA and NaOH are added to the reaction mixture, as taught by Raehse. Raehse teaches that additional reaction stages are effective in producing a product which is highly substituted. Although Raehse teaches reaction of cellulose instead of inulin, the skilled artisan would expect that methods which are effective for substitution of cellulose would also be effective for substitution of inulin

because both are polysaccharides, and both contain primary and secondary hydroxyl groups which are the reactive sites. Further, the reaction conditions used by Verraest for the carboxymethylation of inulin are similar to the reaction conditions used by Raehse: reaction of the polysaccharide, monochloroacetic acid, and sodium hydroxide at elevated temperature. Thus, the skilled artisan would have a reasonable expectation of success in preparing a carboxyalkylinulin via modification of Varraest's method as discussed above. Although Varraest and Raehse are silent with respect to the pH of the reaction mixture, amounts of alkali are described, and the pH is a function of the amount of alkali added. Thus, the skilled artisan would have sufficient guidance to carry out the modified reaction as discussed above and to optimize reaction conditions, which is well within the skill of the skilled artisan.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to LAYLA BLAND whose telephone number is (571)272-9572. The examiner can normally be reached on Monday - Friday, 7:00 - 3:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anna Jiang can be reached on (571) 272-0627. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Shaojia Anna Jiang/
Supervisory Patent Examiner, Art Unit 1623

/Layla Bland/
Examiner, Art Unit 1623

Notice of References Cited	Application/Control No. 10/587,878	Applicant(s)/Patent Under Reexamination RAAIJMAKERS ET AL.	
	Examiner LAYLA BLAND	Art Unit 1623	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-4,507,474	03-1985	Raehse et al.	536/97
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Verraest et al. Carbohydrate Research 271 (1995) 101-112.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

ELSEVIER

Carbohydrate Research 271 (1995) 101–112

CARBOHYDRATE RESEARCH

Carboxymethylation of inulin

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Abstract

Inulin was carboxymethylated in aqueous alkaline medium with monochloroacetic acid as the reagent. The degree of substitution of the reaction product was determined by titration, LC analysis and ^{13}C NMR spectroscopy. Carboxymethylinulin with a degree of substitution between 0.2 and 1 was obtained depending on the molar ratio of inulin–monochloroacetic acid. Increasing the concentration of the reaction mixture and lowering the reaction temperature resulted in higher selectivities towards carboxymethylinulin. Determination of the molecular weight distribution was performed by GPC and by multi-angle laser light scattering. Carboxymethylation caused little or no degradation of the chain length of the starting material.

Keywords: Fructan; Polyelectrolyte; Monochloroacetate; ^{13}C NMR spectroscopy; GPC

1. Introduction

Inulin, a $(2 \rightarrow 1)$-β-D-fructan with a D-glucose unit at the reducing end (Scheme 1), can be found as a reserve polysaccharide in various plants such as chicory, Jerusalem artichoke and dahlia. The average degree of polymerization (dp) varies from 5 to 30 depending on the plant origin. Recently, inulin became commercially available.

Besides the obvious use of inulin as such or as a source for D-fructose, there is a need of synthetic methods for the conversion of inulin into other useful products. A possible method, carboxymethylation, is now reported.

Carboxymethylation is a well known derivatisation process for polysaccharides, giving products in which primary and/or secondary alcohol groups are etherified with carboxymethyl groups. The derivative obtained is a polyelectrolyte and can be applied in

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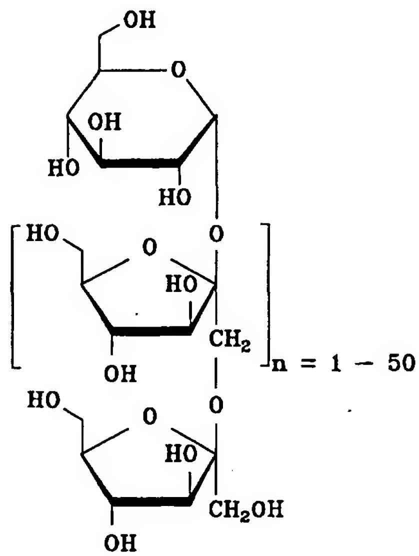

Scheme 1. Inulin.

a wide variety of fields, e.g. as dispersing agent or as metal ion carrier. Other advantages of carboxymethylation are the ease of processing, the low cost of the chemicals and the non-toxicity of the products. An example is carboxymethylcellulose (CMC), which was described for the first time by Jansen (1920) [1,2] and which is an important industrial material at present. The estimated world production in 1991 was about 300,000 tons per year. CMC is used as anti-redeposition agent in detergents (26%), in the oil (20%), paper (18%), textile (6%) and mining industry (5%), as thickener in foods and in pharmaceutical preparations (17%) [3]. Besides cellulose, other polysaccharides such as starch [4,5], guar [6] and mono- and di-saccharides such as sucrose, lactose, D-galactose and D-glucose [7–10] have been carboxymethylated.

2. Experimental

Materials.—Two types of inulin were used. The first type (type I), with an average dp = 30, was obtained from E. Merck (Darmstadt, Germany). The second type (type II), isolated from chicory root, was a gift from Suiker Unie (Roosendaal, The Netherlands) and is commercially available under the name "Fibruline". The average dp is 10.

The dp of inulin was established by LC analysis [column: Dionex Carbopac PA1; detection: Dionex PED 1 pulsed electrochemical detector; eluent: a solvent gradient starting with 70% solvent A (0.1 M NaOH) and 30% solvent B (0.1 M NaOH, 0.5 M NaOAc) and ending with 100% solvent B]. While type I inulin is essentially monosaccharide-free, type II inulin contains 5.4% of monosaccharides (D-glucose and D-fructose) and 4.5% of sucrose.

Carboxymethylation.—Method (a). In a 100-mL round-bottom vessel, inulin (3.4 g, 20 mmol monomeric units) was dissolved in water (25 mL). To this solution,

monochloroacetic acid (MCA) and NaOH were added in a molar ratio of 1:2.0 to 1:2.1. The solution was heated to the reaction temperature and was stirred magnetically for 5 h. After cooling, the reaction mixture was neutralised by addition of a 2 M HCl-solution. The required amount of HCl corresponded to the excess of NaOH at the beginning of the reaction (0 to 0.1 equiv.).

Method (b). Inulin (410 g, 2.5 moles) was mixed with water until a kneadable paste was formed. A 50% NaOH solution (1 mole) was added and the mixture was kneaded for 1 h. After that the sodium salt of MCA (117 g, 1 mole) was mixed thoroughly with the paste. The mixture was heated at 70° C during 3 h. A syrupy product was obtained.

Isolation of the product.—The neutralised reaction mixture was concentrated under reduced pressure to a vol of about 10 mL. The resulting mixture was poured into 100 mL well-stirred absolute MeOH. The sodium salt of carboxymethylinulin (CMI) precipitated as a white solid. Sodium glycolate, formed by hydrolysis of MCA, remained in soln. The residue was filtered off, washed with absolute MeOH and dried under reduced pressure. Finally, NaCl and traces of glycolate and MeOH were removed by membrane filtration (UTC 60, Toray Industries, Inc., Tokyo, Japan) at a pressure of 20 bar. The solution was freeze-dried to yield pure (> 95%) CMI. The product had a slightly brown color.

Determination of the degree of substitution (ds).—The ds of CMI was determined by titration of the carboxylic acid groups, by LC analysis and by ^{13}C NMR spectroscopy.

For the titration of the carboxylic acid groups, the sodium salt of CMI was converted into the acid form by treatment with an acidic cation exchange resin (Dowex 50X8–100, H^+) and subsequent freeze-drying. A portion of the carboxymethylated product (100 mg) was dissolved in 20 mL of distilled water. The carboxylic acid content was then determined by back-titration with 0.1 N HCl after addition of a known amount of NaOH.

In addition, LC analysis was used for the determination of the ds of CMI. To that end, CMI was hydrolysed to monosaccharides by heating (70° C) in aqs soln at pH 1.5 for 1 h. After neutralization, the solution was analysed by LC [column: Phenomenex (Bester, Amstelveen, the Netherlands), Rezex Organic Acid, 300 × 7.8 mm; eluent: 0.01 M trifluoroacetic acid; 60° C; flow rate: 0.6 mL/min; RI and UV_{215} detection]. The products were identified by LC-MS analysis using the same column and conditions. In this case, the LC system was coupled to a VG 70-SE mass spectrometer. Monosubstituted, disubstituted and non-substituted monosaccharides were found. The carboxylic acid content of the product was calculated from the integrals of the peaks.

The last technique used for determination of the ds was ^{13}C NMR spectroscopy. The spectra were recorded on a Varian VXR-400 S spectrometer using D_2O as solvent and *tert*-butanol as internal standard. ^{13}C NMR spectra were measured quantitatively (relaxation delay: 30 s.; pulse angle: 45°; decoupler on during acquisition). The carboxylic acid content was calculated from the peak integrals of carboxylic acid groups (δ 179 ppm) relative to those of C-2 of inulin (δ 104 ppm).

Gel-permeation chromatography (GPC).—The molecular weight distribution of inulin and CMI was determined by GPC on a Bio-Gel P-6 (Bio-Rad) column, using the following conditions: column diameter: 0.8 cm; column length: 65 cm; eluent: 0.02 M NH_4HCO_3; flow rate: 6 mL/h; sample: 100 mg/0.5 mL; detection: RI. Fractions of 3

mL were collected with an automatic fraction collector. The fractions containing organic material were combined, hydrolysed and analysed by LC as described above. The column was calibrated by fractionation of D-fructose, nystose (GF_3) and inulin with an average dp = 30 (GF_n).

GPC coupled with multi-angle laser light scattering.—A high-performance GPC system (GPC 150 C, Waters) was coupled with a multi-angle laser light scattering (m.a.l.l.s.) detector (Dawn-DSP-F, Wyatt Technology). A refractometer was used as a concentration-sensitive detector. The samples were chromatographed on two GPC-columns coupled in series (TSKgel G3000PW_{XL} and TSKgel G5000PW_{XL}) using 0.1 M $NaNO_3$ (1 mL/min) as eluent. The specific refractive increment (dn/dc) was determined on an interferometric refractometer (Optilab, Wyatt Technology) equipped with a 633 nm filter to isolate the wavelength produced by the laser used in the light scattering detector. For inulin and its carboxymethyl derivatives, $dn/dc = 0.131$ mL/g.

3. Results and discussion

Carboxymethylation of inulin.—Inulin (type I) was carboxymethylated by heating an aqueous solution at 95° C for 5 h with monochloroacetic acid (MCA) and sodium hydroxide. The molar ratio MCA-inulin was varied between 0.5:1 and 4:1. The degree of substitution of the isolated reaction products was determined by titration, LC-analysis and ^{13}C NMR. The results are compiled in Table 1.

Since the results obtained by the three analytical techniques agree well, it can be concluded that all are suitable methods for determining the ds of CMI. Under the conditions employed, reaction products with a ds of 0.21 to 1.05 were obtained.

The distribution of the substituents was determined by LC-analysis, separating the group of monosubstituted monosaccharides (3 regioisomers) from the disubstituted (3 isomers) and the non-substituted monosaccharides. An example of a LC-chromatogram is given in Fig. 1. Table 2 shows the distribution of the substituents for the various products.

The product with the lowest ds (ds 0.20) still contains disubstituted monomers, while the product with ds 1.05 still contains 25% of non-substituted monosaccharide residues. When products with increasing ds were prepared, the increase in proportion of disubsti-

Table 1
The ds of CMI as determined by titration, LC-analysis and ^{13}C NMR spectroscopy. Starting materials: inulin (Type I, 20 mmol), MCA and NaOH (molar ratio 1:2) dissolved in 25 mL water; Reaction temperature: 95° C; Reaction time: 5 h

Molar ratio	ds as determined by			MCA
MCA-inulin	Titration	LC	^{13}C NMR	efficiency [a]
0.5	0.22	0.21	0.21	0.42
1	0.40	0.36	0.33	0.36
2	0.66	0.70	0.67	0.34
4	1.05	0.99	1.10	0.25

[a] MCA efficiency is calculated as (average ds / molar ratio MCA-inulin)

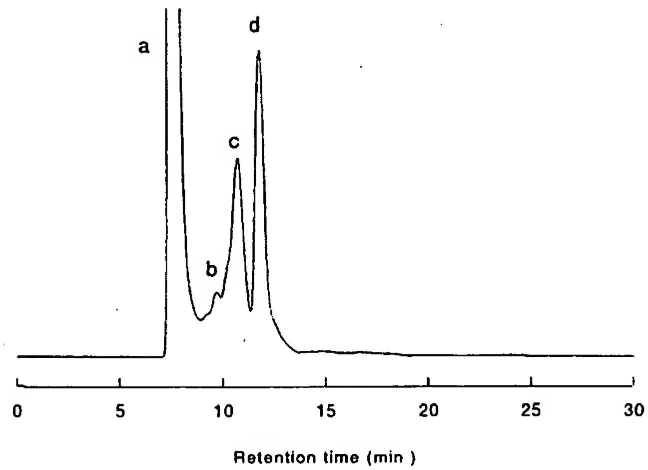

Fig. 1. LC analysis (RI detection) of products formed upon hydrolysis of CMI obtained by carboxymethylation of type II inulin: (a) exclusion peak (inorganic acids and solvent); (b) disubstituted monosaccharides; (c) monosubstituted monosaccharides; (d) non-substituted monosaccharides.

tuted units was larger than that of monosubstituted units (Fig. 2). The conclusion is that carboxymethylation is not selective for a specific position in the monosaccharide unit. In contrast, it has been shown that in cellulose the C-2 position of the glucose units is more reactive towards MCA than the C-6 and the C-3 position [11,12].

An example of a ^{13}C NMR spectrum of CMI (type I inulin as starting material, ds 0.68) is given in Fig. 3. In contrast to the ^{13}C NMR spectrum of inulin, which shows six well-defined peaks due to the six carbon atoms of the fructose units, the spectrum of CMI is more complex because of the substituents at various positions on the fructose units.

The carboxymethylation reactions were monitored by taking samples of the reaction mixture at regular time intervals and analysing them by LC. In Fig. 4, the conversion as a function of time of type II inulin in reaction with MCA and NaOH (1:2:4.2) is given.

Under the conditions used, inulin was rapidly substituted with carboxymethyl groups. A reaction time of less than 2 h appeared to be sufficient to attain the final ds. After this period, only hydrolysis of MCA occurred.

Table 2
Distribution of the substituents in mono-, di- and non-substituted monosaccharide units (in mol %) as determined by LC analysis

	Number of substituents/monosaccharide unit:		
ds of CMI	0	1	2
0.21	83.2	13.0	3.8
0.36	67.6	28.4	4.0
0.68	40.9	48.1	11.0
1.05	24.1	52.5	24.1

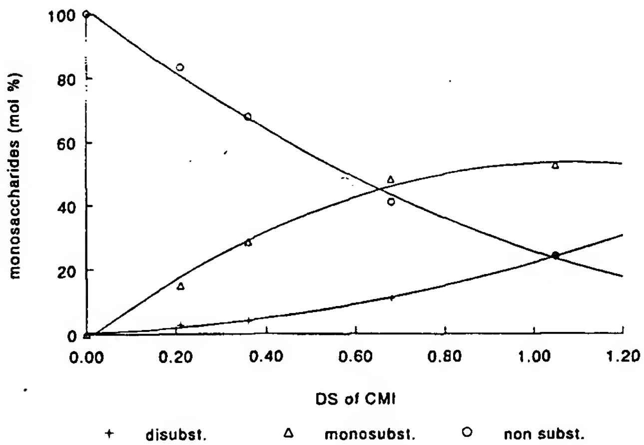

Fig. 2. Monosaccharide composition of carboxymethylated inulin with varying ds.

Efficiency of the carboxymethylation reaction.—During carboxymethylation reactions of polysaccharides in aqueous medium, there is a competition between the carboxymethylation and the hydrolysis of MCA into glycolate. The extent to which the carboxymethylation is favored can be expressed by MCA efficiency (selectivity of the reaction towards carboxymethylinulin).

With standard reaction conditions, the MCA efficiency (calculated as [ds obtained/ratio MCA-inulin]) was higher at lower molar ratio of MCA-inulin (see Table 1).

Besides type I inulin, also type II inulin, nystose and sucrose were carboxymethylated under the conditions described above. A molar ratio of MCA-monosaccharide units of 2:1 was used. For all of the starting materials, a ds of about 0.70 was obtained. It can be concluded that the MCA efficiency does not depend on the chain length of the starting materials.

In order to improve the MCA efficiency, the influence of the reaction temperature and the amount of water in the reaction mixture was studied. In a first series of experiments inulin was carboxymethylated with 2 equiv MCA at three temperatures (95, 75 and 55° C). The volume of water used was 25 mL. The reactions were monitored by taking samples of the reaction mixture and analysing them by LC. The conversions of MCA and the selectivities towards carboxymethylinulin (calculated as [carboxymethyl groups in the product] / ($[MCA_{start}]$ – [MCA]) are given in Table 3. Decreasing the reaction temperature results in a dramatic decrease of the reaction rate. The MCA efficiency was found to be somewhat higher at lower reaction temperatures.

In a second series of experiments, the influence of the water content of the reaction mixture was studied (Table 4).

Decreasing the water content in the reaction mixture has a great influence on the MCA efficiency of the carboxymethylation. When the amount of water was reduced by a factor of five, the selectivity was twice as high. Carboxymethylinulin with the same ds was thus obtained with half the quantity of MCA and sodium hydroxide. The importance

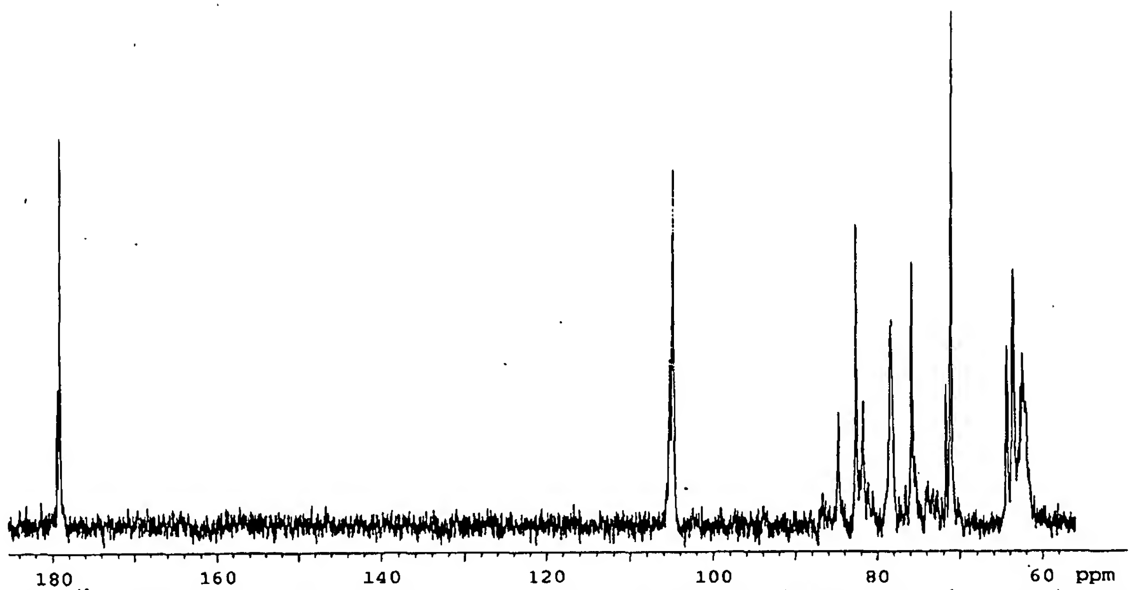

Fig. 3. ^{13}C NMR spectrum of CMI (ds 0.68, prepared with type I inulin) in D_2O (100.572 MHz; 25° C; pH 8.5). Peak assignment: δ 31.2 ppm: reference (*tert*-butanol); δ 62.4 – 64.4 ppm: C-1 and C-6; δ 71.0 – 71.7 ppm: $\mathit{C}H_2COO^-$; δ 75.8 – 88.0 ppm: C-3, C-4 and C-5; δ 104.8 – 105.3 ppm: C-2; δ 179.1 – 179.5 ppm: $CH_2\mathit{C}OO^-$.

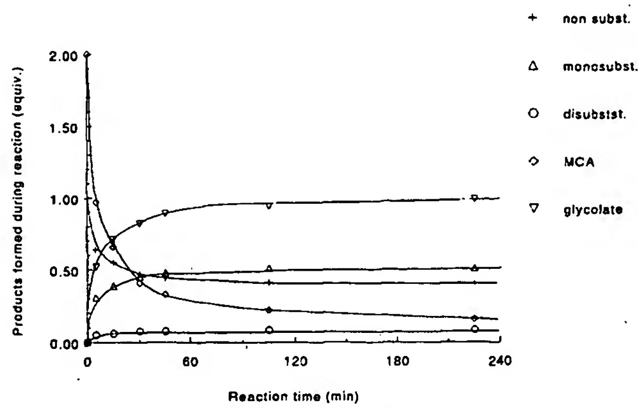

Fig. 4. Conversion of type II inulin (20 mmol) in a carboxymethylation reaction with MCA and NaOH (molar ratio 1:2:4.2) dissolved in 25 mL water at 95° C. Mono-, di- and non-substituted monosaccharides were formed upon hydrolysis of inulin and CMI.

of using low water contents has also been found for the carboxymethylation of cellulose [13,14]. In conclusion, it can be stated that the reaction temperature influences mainly the reaction rate. Decreasing the water content in the reaction mixture results in higher MCA efficiencies of the carboxymethylation process.

Neither the temperature nor the concentration of the reaction mixture influenced the distribution of the substituents in the products. The relative proportions of di-, mono- and non-substituted units in the end products corresponded to the curves presented in Fig. 2 (data not shown).

In a larger scale experiment, inulin was carboxymethylated successfully using a minimum quantity of water (experimental method b). From LC-analysis, it was concluded that the reaction product was CMI with a ds of 0.24. The conversion of MCA was 73% and the selectivity towards CMI was 81%. Sodium monochloroacetate, sodium glycolate, and sodium chloride were removed by membrane filtration as described before. With this procedure, it was possible to prepare a large amount of CMI using a small reactor volume. The MCA efficiency of the reaction was very high and, in consequence, only a small amount of by-product (glycolate) was formed.

Analysis of molecular weight distribution.—Inulin is a mixture of polysaccharides with different chain lengths. The dp varies from 1 to 50, with an average dp depending on the plant it originates from.

Table 3
Effect of the reaction temperature on the conversion of MCA and the selectivity towards CMI. Starting materials: inulin (Type II, 20 mmol), MCA (40 mmol) and NaOH (80 mmol) dissolved in 25 mL water

Temperature (° C)	Reaction time for 85% conversion (h)	Selectivity (%)	ds of CMI
55	24	43	0.73
75	5	39	0.66
95	2	38	0.65

Table 4
Effect of the water content of the reaction mixture on the conversion of MCA and the selectivity towards CMI. Starting materials: inulin (Type II, 20 mmol), MCA and NaOH (molar ratio 1:2) dissolved in water; Reaction temperature: 75° C; Reaction time: 5 h. In all cases the conversion of MCA was 85%

Volume water (mL)	Molar ratio MCA:inulin	Selectivity (%)	ds of CMI
25	2 : 1	39	0.65
10	2 : 1	52	0.90
5	1 : 1	78	0.65

The average dp can be easily determined from the ratio of D-glucose:D-fructose after acidic or enzymatic hydrolysis of inulin [15]. However, this technique cannot be used for modified inulin and it is not possible to establish degradation or cleavage of the polysaccharide chains. Another disadvantage is that no information can be obtained about the molecular-weight distribution of the products. Therefore, the molecular-weight distribution of inulin and its carboxymethyl derivatives was determined by gel-permeation chromatography (GPC) on a polyacrylamide gel (Biogel P-6) [15–19]. In Fig. 5 the

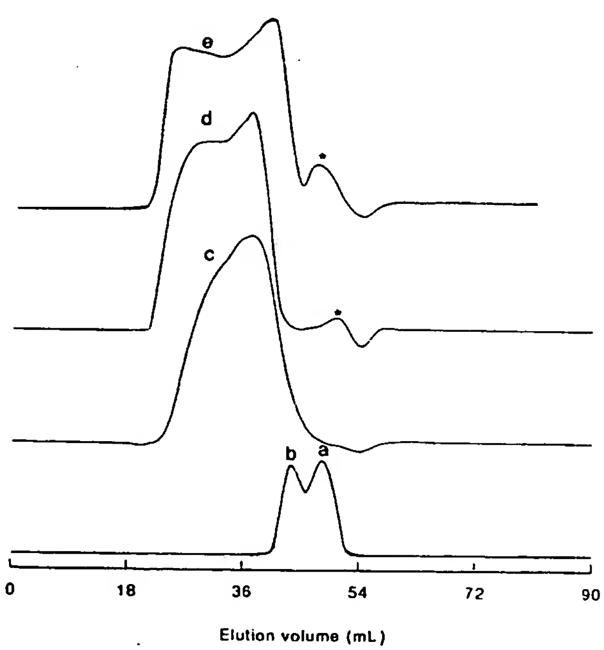

Fig. 5. GPC separation on Biogel P-6 (RI detection) of fructose (a), nystose (b), inulin (type I) (c), CMI with a ds of 0.36 (d) and CMI with a ds of 0.68 (e). Chromatographic conditions as described in the text. The small peaks marked with a " * " contained no organic material and are probably due to small amounts of inorganic salts.

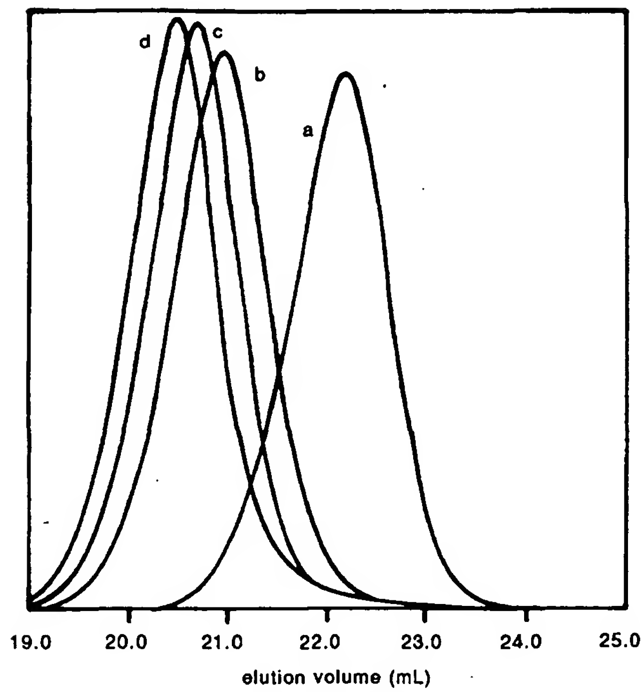

Fig. 6. Elution profiles from high performanced GPC (RI detection) of inulin (type I) (a), CMI with a ds of 0.68 (b), CMI with a ds of 0.42 (c) and CMI with a ds of 1.05 (d).

elution profiles of nystose (GF_3), fructose, inulin (type I) and CMI with a ds of 0.68 and 0.36 are given.

With the GPC system used, type I inulin, nystose and fructose were satisfactorily separated. Low molecular weight material can thus easily be distinguished from inulin and its derivatives. Due to chain stiffening and extension because of the electrostatic repulsion of carboxymethyl groups, CMI eluted at a slightly lower elution volume than inulin.

The fractions obtained by the GPC fractionation were hydrolysed and analysed with

Table 5
Average molecular weights of inulin and CMI from GPC-MALLS experiments

Material	Average M_w [a] (± 10 %)	Calculated [b] av. M_w
inuline (type I)	4300	4878
CMI (ds 1.05)	5300	7398
CMI (ds 0.68)	5700	6510
CMI (ds 0.42)	5300	5886

[a] The values represent averages of 2 runs. [b] Average molecular weights were calculated assuming an average dp of inulin of 30 as was established with LC (see section materials).

LC in order to determine the ds of carboxymethylated material as a function of the molecular weight. The ds was found to be the same for all the fractions containing CMI. This confirms that the selectivity of the carboxymethylation reaction does not depend on the chain length of the oligosaccharides.

Using gel-permeation chromatography no absolute value for the average molecular weight of CMI could be determined. The introduction of carboxymethyl groups affects the hydrodynamic volume of the polymer and direct comparison of a charged and and non charged material is not obvious. Therefore, some additional molecular weight determination was performed using multi-angle laser light scattering coupled with GPC (GPC-MALLS) [20]. With this technique, on-line determination of the molecular weight of the eluting material from the GPC-column was possible. The elution profiles, as detected with RI, are shown in Fig. 6. Similar to the preparative GPC profiles (Fig. 5), the carboxymethylated derivatives eluted at a lower elution volume as compared to inulin. The MALLS response of the inulin sample showed a distinct prepeak at a retention volume coinciding with the exclusion limit of the column. This prepeak is due to a small amount ($< 1\%$) of high molecular weight impurities[20] and was not taken into account for the molecular weight calculations. These impurities were not found in the samples of carboxymethylated inulin, which means that they were successfully removed during the work-up procedure of the product. The average molecular weights as obtained from the MALLS response were slightly lower (about 10%) than the calculated values for inulin as well as for CMI (Table 5). Only for CMI with the highest ds (ds 1.05) the deviation from the calculated value was somewhat higher than for the other products, which could point to some degradation due to the high sodium hydroxide concentration. However, this degradation is not detrimental for the application of the polymeric material. It can be concluded that almost no chain length degradation occurs during the carboxymethylation reaction.

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